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Recombination in *Bact. coli K* 12: Unidirectional Transfer of Genetic Material

THE development of nutritionally independent prototroph colonies from mixed cultures of doubly dependent mutant strains of *Bact. coli K* 12 was first demonstrated in 1946[1]. Back mutation to prototrophism did not occur when the mutants were cultured separately. Since the pattern of unselected marker characters in prototrophs was usually different from that in either mutant, the phenomenon was clearly due to genetic recombination. The incompetence in recombination of culture filtrates (unlike type transformation in *Pneumococcus*), and recent evidence for the occasional occurrence of diploid heterozygous prototrophs[2], strongly support the current theory that the genetic transfer is mediated by sexual conjugation. Attempts to reproduce the phenomenon in other strains and species have failed, though successful out-crossing of *K* 12 mutants with a strain of *Bact. acidi lactici* has been reported[3].

In the following experiments, *K* 12 mutants 58–161, requiring biotin and methionine, and *W* 677, requiring leucine, threonine and aneurin, were employed. An attempt was made to investigate the dynamics of recombination by adding streptomycin at intervals to a series of plates of basal medium (plus aneurin) seeded with a mixture of 58–161 and a streptomycin-resistant mutant of *W* 677 (*W* 677/S^r). It was anticipated that the streptomycin would rapidly block the recombination mechanism by inactivating 58–161, while allowing resistant prototroph cells formed prior to its addition to develop into colonies. In practice, the number of prototroph colonies did not differ greatly whether streptomycin was incorporated in the basal medium before plating or was added up to four hours later. Since similar results were obtained when the mutants were mixed for the first time during plating, the occurrence of recombination in mixtures before contact with streptomycin was excluded. Either prototrophs arose before the action of streptomycin on the sensitive mutant became effective, or else those functions of the cell affected by streptomycin were not involved in recombination.

Logarithmic-phase broth cultures of 58–161 were treated with either 1,000 or 2,000 μgm./ml. streptomycin for periods up to 18 hr., under conditions optimal for bactericidal effect. Washed saline concentrates of treated cultures (58–161/S^t), although frequently sterile, invariably stimulated prototroph

formation when mixed with *W* 677/S^r on basal medium containing 200 μgm./ml. streptomycni. Whenever streptomycin treatment failed to produce sterility, control reconstruction experiments showed that at least a thousand times as many untreated 58–161 cells as those which had survived treatment were necessary for prototroph formation under similar conditions.

Mixtures of streptomycin-treated *W* 677 (*W* 677/S^t) and 58–161/S^r, on the other hand, invariably failed to produce prototrophs, although comparable recombination-rates were given by the mixtures (*W* 677/S^r + 58–161) and (58–161/S^r + *W* 677). In these experiments streptomycin was not incorporated in the basal medium, since previous analysis of proved prototrophs had shown that about 95 per cent carried the S^r or S^s character of *W* 677. The clear-cut distinction between 58–161/S^t and *W* 677/S^t in ability to participate in recombination was shown to be independent both of the presence of the S^r character in the complementary mutant and of the basal medium environment. Thus (58–161/S^t + *W* 677) produced prototroph colonies on every occasion, whether cultured directly on basal medium or initially on nutrient agar. The mixture (*W* 677/S^t + 58–161), however, failed to do so repeatedly on basal medium and, in a single experiment, when seeded on nutrient agar.

It is unlikely that sensitive cells which have been acted upon for 18 hr. by very high concentrations of streptomycin can still participate in cytoplasmic fusion in the continued presence of the drug. Moreover, if conjugation under these conditions was possible for 58–161/S^t, it might also be assumed for *W* 677/S^t. Yet suspensions of the latter are inactive in recombination. It is more probable that recombination is mediated by genetic elements, extruded by the viable cell, which adhere to the cell wall and which, like viruses, are unaffected by streptomycin. Thus, the dead cell could serve merely as a passive carrier for its genetic elements after their expulsion. The incompetence of *W* 677/S^t becomes intelligible only if we suppose that the role of *W* 677 is primarily the vital one of accepting genes and incorporating them into its genetic structure.

It is known that symbiotic bacterial viruses can transfer hereditary characters to heterologous strains[4], and that *K* 12 harbours a virus which can be liberated by small doses of ultra-violet light[5]. The known facts of recombination, and especially its marked enhancement by small sub-mutagenic doses of ultra-violet light[6] and the presumptive one-way transfer of the genetic agent from 58–161 to *W* 677, suggest the possibility that this agent may be a virus. The

existence of a latent virus in 58–161 has been unmasked by *X*-radiation (personal observation).

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